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Research Article

Didymocarpus pingyuanensis (Gesneriaceae), a new species endemic to Danxia landscape from Guangdong Province, China, and two new combinations in *Didymocarpus*

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Abstract

Didymocarpus pingyuanensis, endemic to the Danxia landscape in Pingyuan County, Guangdong, China, is described and illustrated here. This species can be distinguished from other members of *Didymocarpus* sect. *Heteroboea* by its calyx deeply 5-lobed to about three quarters of its length. The phylogenetic position of the new species within *Didymocarpus* was examined using nuclear ribosomal internal transcribed spacer (ITS) sequences. Based on phylogenetics analysis and morphological evidence, we propose two new combinations, elevating the two varieties to species level, namely *D. yinzhengii* and *D. gamosepalus*.

Key words: *Didymocarpus heucherifolius* var. *gamosepalus*, *Didymocarpus heucherifolius* var. *yinzhengii*, flora of Danxia, taxonomy

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Introduction

Didymocarpus Wall. was once a large genus with approximately 200 species (Weber and Brutt 1998). Recent molecular phylogenetic studies and morphological revisions of *Didymocarpus* have led to a reduction in the estimated number of species from 200 down to 60–80, with some species being transferred to *Henckelia* Spreng., *Hovanella* A.Weber & B.L.Burtt, *Petrocodon* Hance (Weber and Brutt 1998; Weber et al. 2000, 2011; Möller and Clark 2013).

For species of this genus in China, Wang et al. (1990) classified them into two sections: sect. *Didymocarpus* (herbs with stems) and sect. *Heteroboea* W.T.Wang auct. non Benth (herbs without stems). Sect. *Heteroboea* were initially defined by morphological characters, but on the basis of recent systematic results and morphological comparison, four species have been assigned to the genus *Petrocodon*, these species are *P. bonii* (Pellegr.) A.Weber & Mich.

Möller, *P. mollifolius* (W.T.Wang) A.Weber & Mich.Möller, *P. niveolanosus* (D. Fang & W.T.Wang) A.Weber & Mich.Möller, *P. hancei* (Hemsl.) Mich.Möller & A.Weber (Weber et al. 2011). Recently, four new taxa within this section were discovered and published: *Didymocarpus dissectus* F.Wen, Y.L.Qiu, Jie Huang & Y.G.Wei (Wen et al. 2013) from Fujian Province, *D. heucherifolius* Hand.-Mazz var. *yinzhengii* J.M.Li & S.J.Li (Li and Li 2014) from Hunan Province, *D. heucherifolius* Hand.-Mazz var. *gamosepalus* Xin Hong & F.Wen (Xu et al. 2019) from Guangdong, China, and *D. lobulatus* F.Wen, Xin Hong & W.Y.Xie (Xie et al. 2020) from Zhejiang, China. In addition, *D. subpalmatinervis* W.T.Wang, which was placed in sect. *Heteroboea* has been transferred to *Petrocodon* as a new combination with *P. subpalmatinervis* (W.T.Wang) F.Wen & Z.L.Li after a thorough study (Li et al. 2023). Thus, before the completion of the revision work for this article, there were eight species and two varieties in sect. *Heteroboea*, all of which are endemic to China.

During a field investigation of Danxia landscapes in Pingyuan County, Guangdong, in April 2023, we encountered a *Didymocarpus* species in bloom and confirmed its classification within sect. *Heteroboea* of this genus as it was stemless herb (Wang et al. 1990). However, it differs from all known species in this section by having a calyx that is deeply 5-lobed to about three quarters of its length. After thorough morphological comparisons using herbarium specimens, digital images, and relevant literature on other similar species, we concluded that this plant represents an undescribed species. Here, the putative species is described and illustrated based on morphological observations and compared with closely related species. Additionally, we used nuclear DNA internal transcribed spacer (ITS) to reconstruct the phylogeny to evaluate the phylogenetic position of *Didymocarpus pingyuanensis*.

Material and methods

Morphological study

We used a micrometer and a stereomicroscope to observe and measure the morphological traits of the putative species. Morphological comparisons between *Didymocarpus pingyuanensis* and its related species were based on dry specimens we collected, relevant literature (Wen et al. 2013; Li and Li 2014; Xu et al. 2019; Xie et al. 2020), as well as digital images on the Chinese Virtual Herbarium (https://www.cvh.ac.cn/) and the China Field Herbarium (https://www.cfh.ac.cn/). Morphological observation was conducted in the Herbarium of Sun Yat-sen University (SYS).

Taxon sampling and molecular analysis

The ITS region was used for examining the phylogenetic position of the putative species. During May 2023, we collected 6 taxa belonging to sect. *Heteroboea* for this study. These taxa included *Didymocarpus cortusifolius* (Hance) H.Lév., *D. salviiflorus* Chun, *D. lobulatus*, *D. heucherifolius* var. *heucherifolius* Hand.-Mazz, *D. heucherifolius* var. *yinzhengii*, *D. heucherifolius* var. *gamosepalus*. Except for *D. heucherifolius* var. *gamosepalus*, all species were collected from their type localities. The population of *D. heucherifolius* var. *gamosepalus* at its type locality

has gone extinct due to human disturbance caused by nearby village activities. Consequently, we were unable to find it at its type locality in Pingyuan County, Guangdong. Instead, we collected specimens from Zijin County, Guangdong. Voucher specimens were deposited in SYS. We sequenced their ITS gene sequences to verify the molecular differences; the sequences have been uploaded to GenBank. Thirty-eight species of *Didymocarpus* and related genera with ITS gene sequences in NCBI GenBank were downloaded for analysis. In total, we got 45 ITS gene sequences of 45 taxa, GenBank accession numbers were followed after their Latin name in the phylogenetic tree. These taxa encompass three *Gyrocheilos* W.T.Wang species, one *Allocheilos* W.T.Wang species, one *Raphiocarpus* Chun species, six *Primulina* species, three *Petrocodon* species, nineteen species from *Didymocarpus* sect. *Didymocarpus* and nine from *Didymocarpus* sect. *Heteroboea*. *Sinningia incarnata* (Aubl.) D.L.Denham and *S. tubiflora* Fritsch were selected as outgroups. All but one species of *Didymocarpus* sect. *Heteroboea* were included in this study. The excluded species was *Didymocarpus reniformis* W.T.Wang, which was not located during multiple expeditions, and only imprecise locality data were available.

Total DNA was extracted from silica-gel-dried leaves using the modified cetyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle 1987). Polymerase chain reaction was carried out based on the program setting as proposed by Lee et al. (2022) using the universal primers, ITS1 and ITS4 (White et al. 1990). The ITS sequence was aligned with Clustal W which is embedded in MEGA-11 (Tamura et al. 2021). The sequences in the alignment were manually adjusted. Phylogenetic trees were reconstructed using the maximum likelihood (ML) methods and Bayesian inference (BI) methods via MEGA-11 (Tamura et al. 2021) and MrBayes v3.2.1 (Ronquist et al. 2012). The optimum DNA substitution model calculated using the "Find best DNA/Protein Models (ML) function embedded in MEGA-11 was Kimura 2-parameter model (K2) with Gamma Distributed With Invariant Sites (G+I) (=K2+G+I). All branch nodes were calculated with 1000 bootstrap (BS) replicates. BI analysis employed random starting trees and four Markov chain Monte Carlo (MCMC) simulations were run simultaneously and sampled every 1000 generations for 1 million generations. Bayesian posterior probabilities (PP) were calculated as the majority consensus of all sampled trees with the first 25% discarded as burn-in.

Results and discussion

Molecular analysis

The aligned length of the ITS sequences was 730 bps. The topologies of the Bayesian and ML trees are incongruent. The BI tree is displayed below (Fig. 1), ML tree is displayed in Appendix 1: Fig. A1. The topology indicates that *Didymocarpus* sect *Heteroboea* is paraphyletic, and eight taxa of *Didymocarpus* sect *Heteroboea* belong to clade I (Fig. 1. PP = 1, BS = 100%). In this clade, the putative new species forms a sister group with *Didymocarpus dissectus*, *D. heucherifolius* var. *gamosepalus* and *D. heucherifolius* var. *yinzhengii* (Fig. 1. PP = 1, BS = 81%), while *D. heucherifolius* var. *heucherifolius* belongs to another group (Fig. 1, PP = 1, BS = 81%).

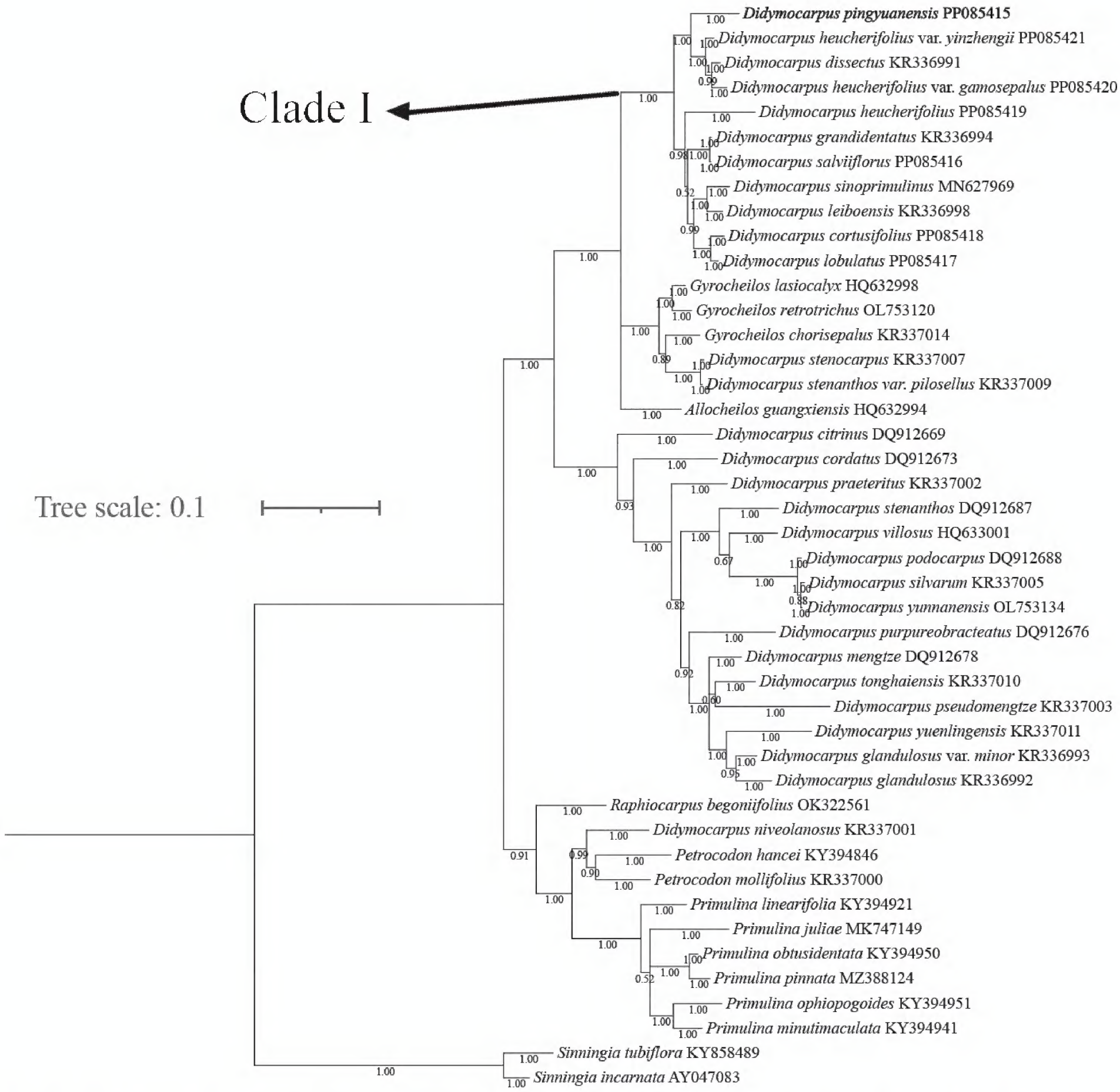

Figure 1. Bayesian inference (BI) tree based on ITS sequences of the new species *Didymocarpus pingyuanensis* and related species. Bayesian posterior probabilities are shown along the branches. The new species described in this study is shown in bold.

Morphological comparison

In *Didymocarpus* sect. *Heteroboea*, most species exhibit similar vegetative characteristics, except for *D. dissectus*, whose leaf margin is irregularly and distinctly 3- or 4-lobed in the distal 1/3–1/2. There are two varieties, *D. heucherifolius* var. *gamosepalus* and *D. heucherifolius* var. *heucherifolius* distributed in Guangdong Province, China. Both *D. pingyuanensis* and *D. heucherifolius* var. *gamosepalus* share the same distribution area, namely Pingyuan County in Guangdong, and both are distributed within the Danxia landscape. During the field investigation, we also found *D. heucherifolius* var. *heucherifolius* in Wuzhishi scenic spot, which is about 50 km from the locality of *D. pingyuanensis*. However, *D. pingyuanensis* can be distinguished from *D. heucherifolius* var. *heucherifolius* by its glabrous corolla.

Furthermore, it can be distinguished from *D. heucherifolius* var. *gamosepalus* by its shorter corolla, and calyx deeply 5-lobed to about three quarters of the calyx length, lobes equal, obovate, apex rounded, rarely cuspidate, overlapping at margin.

Although *Didymocarpus pingyuanensis* and *D. salviiflorus* share calyx lobed and overlapping at margin, however, the former is deeply 5-lobed to about three quarters of the calyx length, contrasting with the latter 5-lobed to about half of the calyx length from the base. Additionally, *D. pingyuanensis* can also be distinguished from *D. salviiflorus* by bracts free, elliptic, and corolla glabrous outside.

Differences between the putative species and its morphologically related species, *Didymocarpus heucherifolius* var. *heucherifolius*, *D. heucherifolius* var. *gamosepalus*, and *D. salviiflorus* are shown in the following identification table (Table 1) and Fig. 2.

Table 1. Morphological differences between the species *Didymocarpus pingyuanensis*, *D. heucherifolius* var. *heucherifolius*, *D. heucherifolius* var. *gamosepalus*, *D. salviiflorus*.

Character	*Didymocarpus pingyuanensis*	*D. heucherifolius* var. *heucherifolius*	*D. heucherifolius* var. *gamosepalus*	*D. salviiflorus*
Shape of calyx	deeply 5-lobed to about three quarters of the calyx length, obovate, overlapping at margin	5-lobed to the base, broadly lanceolate to oblanceolate-linear or triangular	5-lobed from middle to above middle	5-lobed to about half of the calyx length from the base, depressed oblong, overlapping at margin
Bracts	bracts free, elliptic, 5–12 mm, serrate, long ciliate	bracts free, elliptic, 5–10 mm, serrate, long ciliate	bracts free, 4–8 mm, serrate, long ciliate	bracts free to connate, semiorbicular, ca. 5 mm, margin sparsely crenate
Size of corolla	1.8–3.8 cm long	2.5–3.2 cm long	3.6–4.3 cm long	2.5–3 cm long
Indumentum of corolla	glabrous	puberulent	glabrous	puberulent
Staminodes	1.5–4 mm from the base	1–2 mm from the base	1–1.6 cm from the base	3–5 mm from the base

Figure 2. Morphological differences between *Didymocarpus pingyuanensis*, *D. heucherifolius* var. *heucherifolius*, *D. heucherifolius* var. *gamosepalus*, and *D. salviiflorus* **A** *D. pingyuanensis* **B** *D. heucherifolius* var. *heucherifolius* **C** *D. heucherifolius* var. *gamosepalus* **D** *D. salviiflorus*; **1**. front view of corolla; **2**. lateral view of corolla, showing calyx and bracts (Photographers: **A–C** by Qiang Fan **D** by Ling-Han Yang).

Taxonomic treatment

Didymocarpus pingyuanensis **Ling H.Yang, Q.Fan & F.Wen, sp. nov.**
urn:lsid:ipni.org:names:77345325-1
Figs 3, 4

Diagnosis. *Didymocarpus pingyuanensis* is similar to *D. heucherifolius var. gamosepalus* and *D. salviiflorus* in having a similar zygomorphic corolla and pink to pinkish-purple funnel-shaped to tubular corolla tube, but can be distinguished from *D. heucherifolius* var. *gamosepalus* by its corolla size 1.8–3.8 cm long (*vs.* 3.6–4.3 cm long), calyx deeply 5-lobed to about three quarters of the calyx length, apex rounded, rarely cuspidate, overlapping at margin (*vs.* 5–lobed from middle to above middle); from D. salviiflorus, it differs by having calyx 5-lobed to about three quarters of the calyx length (*vs.* 5-lobed to about half of the calyx length from the base), bracts free, elliptic (*vs.* bracts free to connate, semiorbicular), and corolla glabrous outside (*vs.* puberulent outside).

Type. China. Guangdong Province: Meizhou City, Pingyuan Town, 24°32'N, 115°50'E, 491 m a.s.l., 1 April 2023 (fl.), *Qiang Fa, Xing-yue Zhang, Li-juan Liao, Jie-hao Jin, Ling-han Yang DNPC 3352* (holotype: SYS!; isotypes: IBK! IBSC! SYS!)

Description. Acaulescent perennial herb. ***Rhizome*** horizontal, 2–4 cm long, ca. 1 cm in diameter. ***Leaves*** 4–9 basal, clustered at the apex of the rhizome, whorled; leaf blades chartaceous, orbicular-ovate to triangular, 4–11 cm long, 4.5–12 cm wide, apex slightly acute, base cordate, margin lobed (lobes 18–21, irregularly triangular, with irregular teeth), upper surface densely cover with white pilose hairs, green, lower surface with sparsely short hairs and pilose brown hairs confined to the veins, pale green, basal veins 5, palmate; petiole terete, pale green, 2.5–9 cm long, densely covered with pilose brown hairs, ***Cymes*** axillary, usually 3–6 on a stem, pseudodichotomous, scapiform, each 2- to numerous flowered; Peduncle 10–18 cm long, covered with white dense short hairs and sparsely pilose brown hairs; ***Bracts*** free, elliptic, 5–12 mm long, serrate, long ciliate; Pedicel up to ca. 2.5 cm long, with short hairs; ***Calyx*** deeply 5-lobed to about three quarters of the calyx length, lobes equal, obovate, apex rounded, rarely cuspidate, overlapping at margin, 6–9 mm long, 4.5–6 mm wide at the widest part and 2–3 mm wide at the base, lobes margin denticulate, puberulent outside, glabrous inside. ***Corolla*** zygomorphic, pink to magenta, up to ca. 3.8 cm long; glabrous outside, inside with glandular puberulent hairs from the throat to the corolla base and two wide bright yellow strips at the throat; tube funnel-shaped to tubular, 1.2–2.3 cm long, ca. 6.5 mm in diameter at base, ca. 1.1 cm in diameter at throat; limb distinctly 2–lipped, adaxial lip 2-lobed to near middle, 0.6–1 × ca. 1.3 cm, obliquely triangular, abaxial lip 3-lobed to base, lobes rounded or oblong, ca. 0.9 × 1.6 cm, more or less equal. ***Stamens*** 2, adnate to corolla ca. 2 cm above the base of the corolla tube; filaments white, 8–13 mm long, slightly geniculate above base, swollen at middle, glabrous with glandules on the surface; anthers pale yellow, ca. 3 mm long, cohering face to face, white woolly. ***Staminodes*** 3, adnate to 1.5–4 mm above the base of the corolla tube, 0.4–0.8 mm long, white, glabrous. ***Pistil*** 2.2–3 cm long, puberulent; ovary

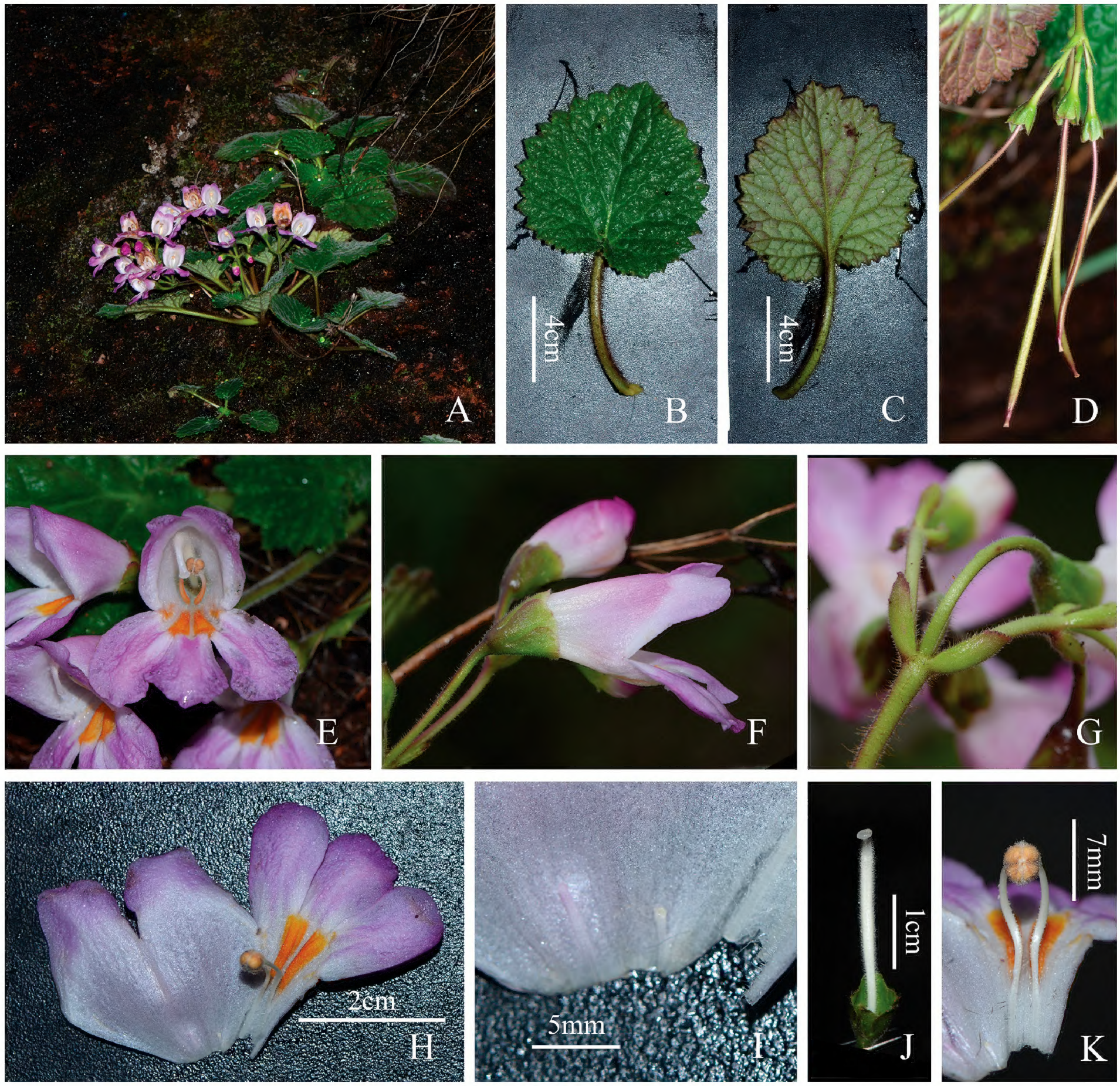

Figure 3. *Didymocarpus pingyuanensis* **A** habitat in flowering **B** adaxial surface view of leaf blade **C** abaxial surface view of leaf blade **D** capsule **E** front view of corolla **F** lateral view of corolla, showing calyx deeply 5-lobed to about a quarter of the calyx length from the base **G** bracts **H** opened corolla **I** staminodes **J** pistil **K** stamens (Photographers: **A–K** by Qiang Fan).

white, cylindrical, puberulent; style ca. 2.3 mm long; stigma 1, cephaloid, centrally sunken, undivided, translucent. ***Capsule*** purplish-red when young, linear-cylindrical, glandular puberulent, up to ca. 9 cm long.

Phenology. The flowering of *Didymocarpus pingyuanensis* is from April to May; and the fruiting is in June.

Distribution and habitat. *Didymocarpus pingyuanensis* so far has only been found on three Danxia landscapes within Pingyuan County. It is locally abundant and endemic to a narrow area near the type locality. This species prefers moist, but sunny cliffs, at an elevation of 100–500 m a.s.l. In Pingyuan, the average temperature is 21.7 °C, and the average annual precipitation is 1637 mm.

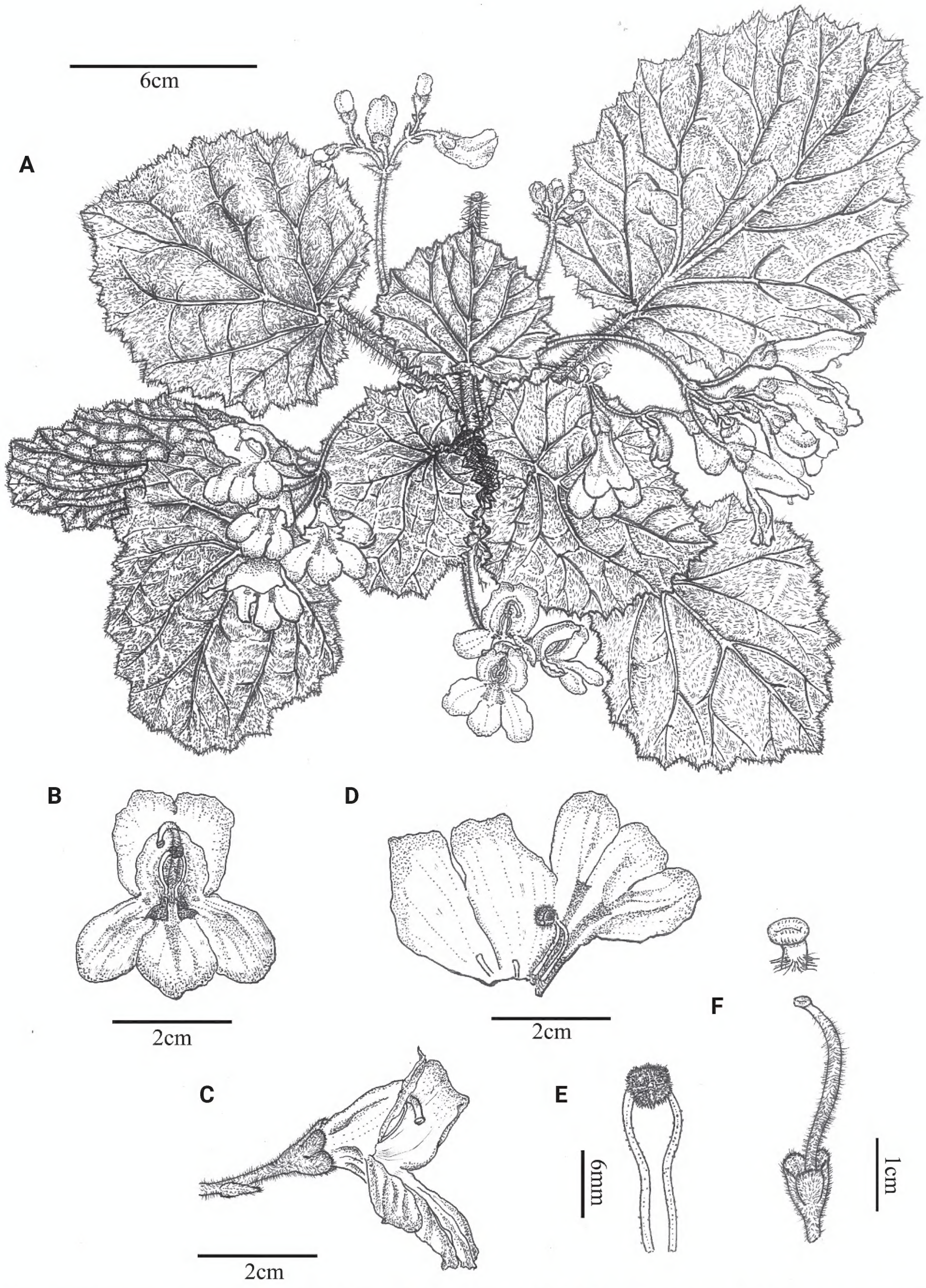

Figure 4. *Didymocarpus pingyuanensis* Ling H. Yang, Q. Fan & F. Wen **A** habit **B** flower in front view **C** flower in lateral view **D** opened corolla, showing stamens and staminodes **E** stamens **F** pistil and stigma (Drawn by Rong-En Wu).

Etymology. The specific epithet refers to the type locality, Pingyuan County, Guangdong Province, China. The Chinese name of the new species is here given as 平远长蒴苣苔 (Píng Yuǎn Cháng Shuò Jù Tái).

Additional specimens examined. *Didymocarpus pingyuanensis* (paratypes): China, Guangdong, Pingyuan: Nantai Mountain, 24°33'N, 115°53'E, 168 m a.s.l., 6 May 2023, *Qiang Fan, Zheng-Fei Li, Ling-Han Yang 20212* (SYS); Nantai Mountain, 24°32'N, 115°50'E, 491 m a.s.l., 7 May 2023, *Qiang Fan, Zheng-Fei Li, Ling-Han Yang 20219* (SYS); Dahebei scenic spot, 24°36'N, 115°49'E, 300 m a.s.l., 7 May 2023, *Qiang Fan, Zheng-Fei Li, Ling-Han Yang 20221* (SYS).

Didymocarpus heucherifolius: China, mountains between Shicheng in southeastern Jiangxi and Ninghua in western Fujian, 1200 m a.s.l., 7 May 1921, *Te-Hui Wang* (IBSC0005130, isotype).

Didymocarpus salviiflorus: China, Zhejiang, Lishui, 19 April 1930, *Guan-Guang Zhong* (00030758, isotype).

Didymocarpus heucherifolius var. *yinzhengii*: China, Hunan, Yongxing, 26°2'28"N, 113°9'8"E, 140 m a.s.l., 13 May 2023, *Qiang Fan, Zheng-Fei Li, Ling-Han Yang 20265* (SYS).

Discussion

Morphologically, *Didymocarpus pingyuanensis* can be distinguished from other species within this genus belonging to *Didymocarpus* sect. *Heteroboea* by calyx deeply 5-lobed to about a quarter of the calyx length from the base, lobes equal, obovate, apex rounded, rarely cuspidate, overlapping at margin. Initially, *Didymocarpus pingyuanensis* seems morphologically like a new variety of *D. heucheifolius* var. *heucheifolius*, because only *D. heucheifolius* var. *heucheifolius* and *D. heucherifolius* var. *gamosepalus* distributed in Guangdong in *Didymocarpus* sect. *Heteroboea*. In addition, *Didymocarpus pingyuanensis* shares corolla glabrous outside with *D. heucherifolius* var. *gamosepalus* and *D. heucherifolius* var. *yinzhengii*, both of them were varieties of *D. heucherifolius*. However, according to the phylogenetic trees, *D. pingyuanensis*, two varieties of *D. heucherifolius* and *D. dissectus* form a sister group, while *D. heucherifolius* var. *heucherifolius* forms a sister group with *D. cortusifolius*, *D. salviiflorus*, *D. lobulatus*, *D. grandidentatus*, *D. leiboensis* and *D. sinoprimulinus*. Thus, we suppose *D. pingyuanensis* is a distinct species (Fig. 1), and elevate the two varieties of *D.heucherifolius* to species level, namely *D. yinzhengii* and *D. gamosepalus*.

New combination

***Didymocarpus yinzhengii* (J.M.Li & S.J.Li.) Ling H.Yang, Q.Fan & F.Wen, comb. nov.**
urn:lsid:ipni.org:names:77345326-1

≡ *Didymocarpus heucherifolius* var. *yinzhengii* J.M.Li & S.J.Li. Phytotaxa 156 (3): 187. 2014.

Type. China. Hunan: near Yongxing County. alt. 300 m, 26°17'10"N, 113°11'25"E, 6 May 2011, *Jia-Mei Li 1105062* (holotype: HEAC!); ibid. *Jia-Mei Li 11501* (paratype: IBK!).

Didymocarpus gamosepalus **(Xin Hong & F.Wen) Ling H.Yang, Q.Fan & F.Wen, comb. nov**
urn:lsid:ipni.org:names:77345327-1

≡ *Didymocarpus heucherifolius* var. *gamosepalus* Xin Hong & F.Wen. PhytoKeys 128: 34. 2019.

Type. China. Guangxi Province, cultivated in the nursery of Gesneriad Conservation Center of China (GCCC), introduced from north of Guangdong Province: Pingyuan County, Meizhou City, growing in rocky crevices at the foot of a calcareous sedimentary rocky hill. 22 February 2019, flowering, *WF20190222-05* (holotype: IBK!; isotype: AHU!)

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Ling-Han Yang participated in field investigation, and taxonomic confirmation and wrote the original draft. Jing-Min Dai assists in the molecular analysis. Jian-Hui Liu, Xue-Zheng Lan participated in field investigation and collected the plants. Qiang Fan and Fang Wen are project leaders and contributed to paper writing.

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Data availability

The newly obtained sequences of *Didymocarpus cortusifolius*, *D. salviiflorus*, *D. lobulatus*, *D. heucherifolius* var. *heucherifoliu*, *D. heucherifolius* var. *yinzhengii*, *D. heucherifolius*

var. *gamosepalus* have been submitted to the NCBI website. The ITS sequence data supporting the findings of this study are available in Fig. 1.

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Appendix 1

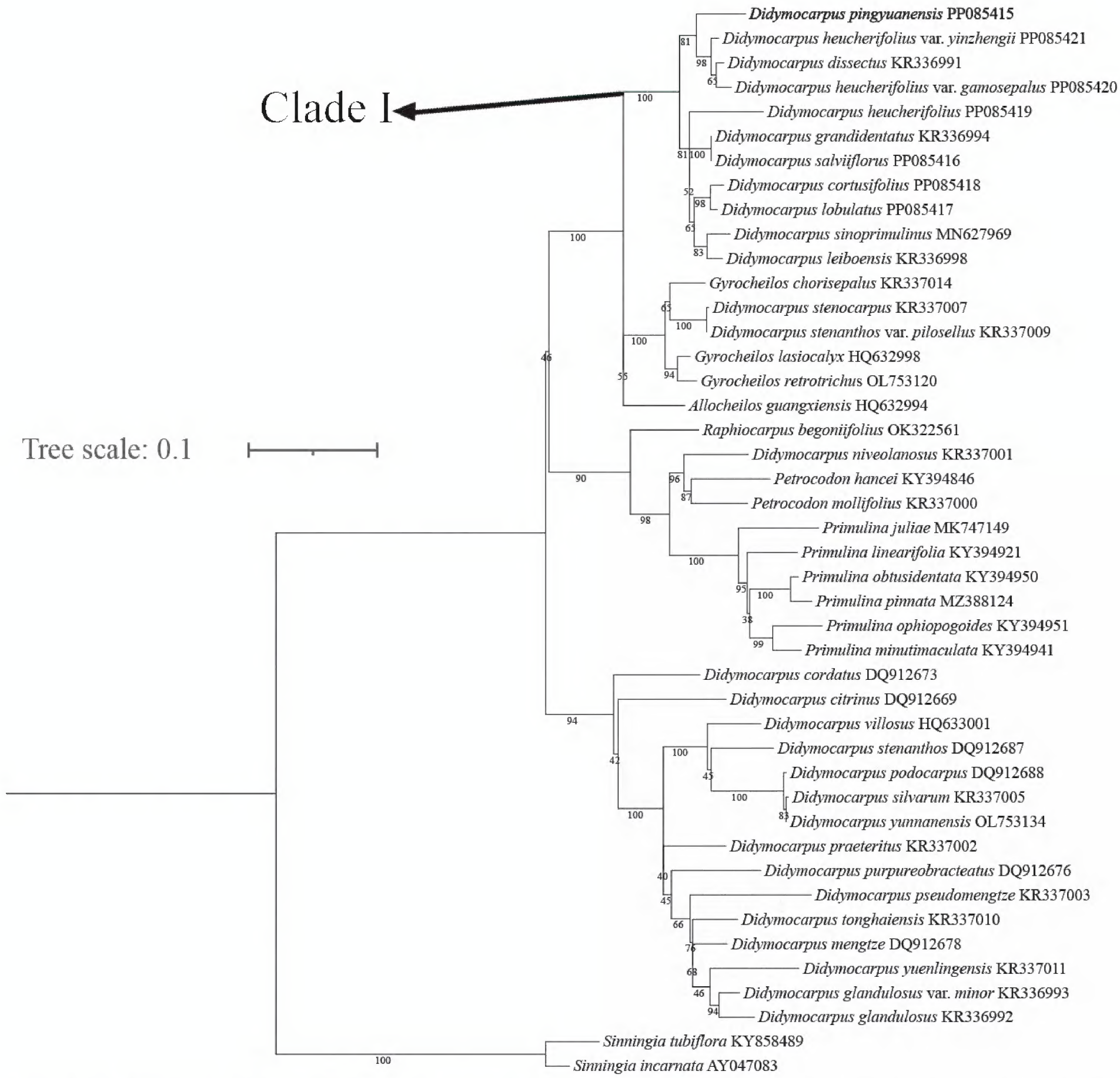

Figure A1. Maximum Likelihood (ML) tree based on ITS sequences of the new species *Didydmocarpus pingyuanensis* and related species. Values of bootstrap support are shown along the branches. The new species described in this study is shown in bold.